Chlorophyll

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On the Exciton Coupling between Two Chlorophyll Pigments in the Absence of a Protein Environment: Intrinsic Effects Revealed by Theory and Experiment

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Abstract: *Exciton coupling between two or more chlorophyll (Chl) pigments is a key mechanism associated with the color tuning of photosynthetic proteins but it is difficult to disentangle this effect from shifts that are due to the protein microenvironment. Herein, we report the formation of the simplest coupled system, the Chl a dimer, tagged with a quaternary ammonium ion by electrospray ionization. Based on action spectroscopic studies in vacuo, the dimer complexes were found to absorb 50–70 meV to the red of the monomers under the same conditions. First-principles calculations predict shifts that somewhat depend on the relative orientation of the two Chl units, namely 50 and 30 meV for structures where the Chl rings are stacked and unstacked, respectively. Our work demonstrates that Chl association alone can produce a large portion of the color shift observed in photosynthetic macromolecular assemblies.*

The absorption wavelengths of chlorophyll (Chl) molecules are modulated by the microenvironment surrounding each pigment molecule. In this way, nature has evolved a method by which the coverage of the optical spectrum and the subsequent transfer of the absorbed energy is optimized,[1,2] leading to photon energy conversion efficiencies of 95% in photosynthetic systems.[3] The harvesting of light energy in photosynthesis is therefore far more efficient than anything thus far developed by our most cutting-edge scientific and technological efforts. Furthermore, small modifications to the basic Chl structure, for example, the replacement of a methyl group in Chl a with a formyl group in Chl b, also lead to some fine-tuning of the absorption spectra.[4]

For decades a great deal of research activity has been directed at understanding precisely how natural systems modulate the absorption energies of Chl species. The highly complex nature of the macromolecular systems involved has, however, always served to complicate such attempts. In recent years, experimental approaches have been joined by theoretical/computational methods, and this has permitted studies at levels of detail that were previously unattainable.[5–12]

Experimental methods have also continued to develop, and recently it has become possible to study absorption processes in Chl molecules free of solvent and other microenvironmental effects. In pioneering experiments, Shafizadeh et al.[13] utilized two-color pump-probe spectroscopy to measure the lowest energy absorption band of neutral Chl a evaporated from spinach leaves. They found the origin band of the Q_y transition to be at 647 nm. Recent action spectroscopy experiments on Chl a tagged with quaternary ammonium cations, in combination with theory, provided an absorption band maximum of similar value (642 nm).[14,15] In this case, the absorption was obtained from the dissociation of the complex, and a calibrated value was determined for the neutral molecule based on the deviation between theory and experiment. Using this same technique, Q-band maxima were obtained for Chl b,[14] and the Soret band was measured for both Chl a and Chl b.[15] Importantly, the large difference in the absorption spectra of Chl a and Chl b was concluded to be an intrinsic effect and not due to local hydrogen-bond interactions with the formyl group of Chl b, for example, clearly demonstrating the advantage of looking at isolated molecules. Compared to literature values for Chl in a variety of natural protein complexes, absorption in vacuo was found to be blue-shifted by 50 nm.[16–19]

With the absorption spectra of bare Chl now well established, we can begin to ask the question of whether shifts that are due to interactions with the protein environment are more or less important than those that are due to excitonic coupling between two or more pigments. This issue was very recently discussed by Baghbanzadeh and Kassal.[20] The interactions basically govern the mechanism for energy

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transfer to the reaction center: For funneling via localized states on the potential energy surface, the protein environment is most important whereas supertransfer requires fully delocalized states where all pigments are electronically coupled. In naturally occurring photosynthetic complexes, there can be significant uninterrupted interfacial regions between Chl molecules, and these presumably play an important part in facilitating energy transfer between pigments. Herein, we have applied the action spectroscopy method used previously on individual Chl pigments to the study of Chl a dimers in vacuo to evaluate the extent to which the close association of chromophores contributes to the spectral shift of Chl in photosynthetic systems.

To study photoabsorption with our approach it is necessary for the chromophore to be charged; however, the direct ionization of a molecule such as Chl in order to produce a charged ion would yield the absorption energies for the anion/cation instead of the neutral molecule. To get around this problem, we have employed our previously developed technique of non-covalently tagging the chromophore with charged species to produce a complex ion.[14,15]

The experimental setup has been described in detail elsewhere.[21,22] Chl a (provided by Sigma–Aldrich) was dissolved in methanol, and salts of either tetramethylammonium ($\mathbf{1}^+$) or acetylcholine ($\mathbf{3}^+$) were added (see Figure 1). These solutions were electrosprayed to produce complexes consisting of chlorophylls and one charge tag. All ions were accumulated in an octopole ion trap for 25 ms. Following extraction from the trap, the ions were accelerated to 40 keV. The charge-tagged complexes were then selected according to their mass-to-charge ratios by an electromagnet and photoexcited by a laser pulse (few nanoseconds long). Photoexcitation led to dissociation of the complex, and the daughter ions were separated according to their kinetic energies (proportional to m/z) using an electrostatic analyzer and counted with a channeltron detector. The light source used was the visible output (420–700 nm) of an optical parametric oscillator (OPO) pumped by a frequency-tripled, Q-switched Nd:YAG laser (EKSPLA). The repetition rate of the laser was 20 Hz, and only every second ion bunch was irradiated. The real photoinduced signal was obtained as the difference between the "laser on" and "laser off" signals. Each ion bunch contained about one hundred charge-tagged Chl dimer complexes.

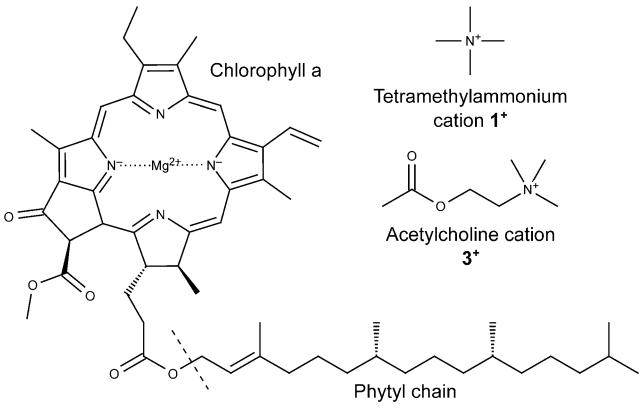

Figure 1. Structures of molecular species employed in the present work. Numbering of the cationic tags chosen to be consistent with our previous related work on Chl spectra.[14,15]

Collision-induced dissociation (CID) experiments were also performed by leaking atmospheric air into the beam line after the mass-selection step and again scanning the electrostatic analyzer (ESA) for daughter ion mass spectrometry. These experiments were done to confirm the identity of the complex, and the corresponding spectra can be found in the Supporting Information.

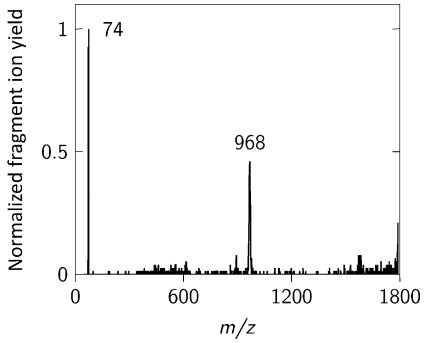

Figure 2. PID mass spectrum of the dimer complex (Chl a)$_2$·$\mathbf{1}^+$ normalized to the highest fragment ion yield. The spectrum was obtained with $\lambda = 420$ nm. Two fragment ions are observed: the charge tag $\mathbf{1}^+$ (m/z 74) and the monomer complex Chl a·$\mathbf{1}^+$ (m/z 968).

Photoexcitation of the dimer cation complexes led to loss of either one Chl a or both Chl a (see Figure 2). Power dependence experiments revealed that at least two photons were needed for dissociation to occur within the instrumental time window of approximately 10 μs. While the complexes are weakly bound, they have many degrees of freedom accounting for dissociation not being a simple one-photon process.

To correct for the variation in laser power across the spectral region, the photoinduced signal was divided by the number of photons at each wavelength, that is, the laser pulse energy was divided by the photon energy, raised to the power of 2.3 (according to the power-dependence measurements, see the Supporting Information). The resulting action spectra associated with each fragment ion are very similar (Supporting Information) and were combined to give a total action spectrum for each charge-tagged dimer (Figure 3). The band maximum occurs at 652 ± 5 nm (1.902 ± 0.015 eV) for both dimer complexes. The spectra shown here were recorded with lower resolution than those in our previous work on Chl monomers[14,15] as the experiment was more difficult owing to much lower ion currents. It is important to note that although the overall band shape slightly depends on the power correction, the band maximum does not within the experimental uncertainty. Hence, our data show that the interaction between two Chl pigments causes a red shift in the absorption band maximum of about 15–25 nm (or 50–70 meV) relative to the monomer charge-tag complexes. There is a second band in the dimer spectrum at higher energy that may be due to the higher-lying exciton state but more work is needed to establish this with certainty.

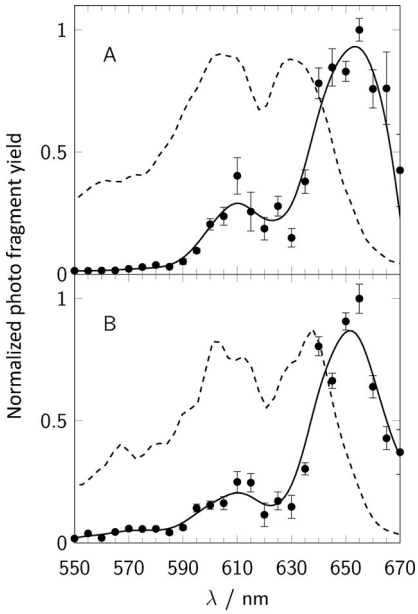

Figure 3. Total 2.3-photon-corrected action spectra for the dimer complexes compared to the monomer complexes (dashed lines). A) Complexes with charge tag $\mathbf{1}^+$. B) Complexes with charge tag $\mathbf{3}^+$. Line smoothing using spline functions was performed because of the low resolution of the dimer spectra. For the purpose of consistency between data sets, the same smoothing was applied to the monomer spectra.

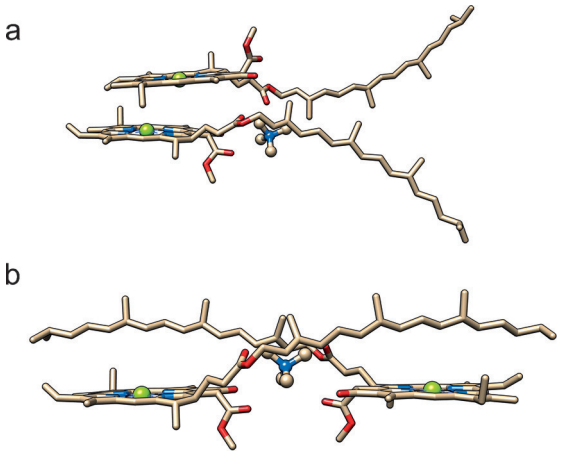

Figure 4. DFT/PBE/Def2-SVP-optimized geometries of the Chl a dimer tagged with $\mathbf{1}^+$ in stacked (a) and linear (b) configurations. Chl a displayed in stick format, charge tag displayed in ball-and-stick format. Hydrogen atoms omitted for clarity.

It is also evident from our spectra that the absorption covers a broad region from 550 nm to 675 nm. This is expected owing to vibronic coupling and the asymmetry of the chromophores (different transition dipoles along each diagonal). Aside from the spectral shift, we observed that the double-maximum character of the monomer spectrum (which is due to vibronic coupling) was lost in the case of the dimer. For now, the origin of this effect remains unclear and requires further study. The band maxima will be compared to the theoretical vertical excitation energies in the following.

The first excitation energies in vacuo for Chl a dimers complexed with a single $\mathbf{1}^+$ charge tag in two different optimized ground-state geometries were calculated using time-dependent density functional theory (TD-DFT; Figure 4).[23] The range-separated CAM-B3LYP functional[24,25] has been found[26] to provide results similar to those obtained with the computationally demanding equation-of-motion coupled-cluster level of theory, and its use ensured consistency with our previous work. The difference in the first excitation energies of chlorophyll (for both the a and b forms) using the charge tags $\mathbf{1}^+$ and $\mathbf{3}^+$ was previously found to be less than 0.03 eV, and it was for this reason that in the present work only the simpler $\mathbf{1}^+$ was employed in the theoretical calculations.[14]

The TD-DFT excitation energies together with the experimental band maxima are given in Table 1. Values for both dimer configurations are given because the energy difference between them was only 0.018 eV at the DFT/CAM-B3LYP/Def2-SVP level of theory, and so both can be expected to be significantly populated at 298 K. The distances between the two magnesium centers are 5.05 Å (stacked configuration) and 19.04 Å (linear configuration). For comparison, the experimental and calculated excitation energies for the monomeric Chl a·$\mathbf{1}^+$ complex from our previous work are also shown.[14] The predicted red shift for the two dimer structures relative to the monomer structure is 30–50 meV, which is close to the measured value of 50–70 meV considering an uncertainty of 15 meV in the experimental data.

The main $Q_y(0,0)$ absorption band in Chl-containing proteins is found between 660 nm and 680 nm but absorption at even longer wavelengths (701–710 nm) is also seen for Chl units in photosystem I.[16–19,27] It is easy to account for absorption in the 660–680 nm region because 1) isolated neutral Chl a and Chl b that are free of charge tags absorb maximally at 642 nm and 626 nm, respectively, according to our previous work, 2) axial ligation has been predicted to give a red shift of 9–19 nm,[28] and 3) the shift that is due to exciton coupling according to our present results is 15–25 nm. The maximum shift would therefore be about 680 nm. However, to account for the absorption at 701–710 nm, it seems that either the two Chl units have to be closer to each other than is the case here (forced together by the protein environment) to increase the coupling—although this seems unlikely as the stacked dimer configuration found in the present work is already close to the lower limit for the intermolecular approach—or in addition to the environmental effect of the protein surroundings, more than two Chl units couple. This is supported by the crystal structures of systems such as the major light harvesting complex (LHC-II) and photosystem I

Table 1: Energies of the experimental band maxima and vertical transition energies for the Chl a dimer. Values in eV with the corresponding absorption wavelengths in nm in parentheses.

	$(\text{Chl a})_2\cdot\mathbf{1}^+$	Chl a·$\mathbf{1}^+$[a]	Δ^{Dimer}
exp.	1.90 (652)	1.97 (629)	−0.07 (+23)
theory (stacked)	2.028 (611)	2.081 (596)[b]	−0.053 (+15)
theory (linear)	2.050 (605)	2.081 (596)[b]	−0.031 (+9)

[a] Values from previous work.[14] [b] Single value for the unique Chl a monomer complex configuration.

from green plants in which close proximity between more than two Chl pigments occurs frequently.[29,30] We aim to investigate the influence of larger pigment assemblies within these protein–Chl complexes in the future by looking at trimers or larger clusters of Chl molecules.

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